sequences. Such second site effects can occur, for example, through the translation of conformational changes to the CDR binding pocket or to the framework regions.

Other effects on binding affinity that will occur due to the combined interactions of two or more amino acid changes within a single variable region species include, for example, the neutralization or augmentation of inherently detrimental changes and the augmentation of beneficial amino acid changes or the augmentation of parent residues. As with the unveiling of beneficial changes and the ability to counteract changes in apparently non-mutable residues, the neutralization and augmentation of amino acid changes within the grafted CDRs or framework region by second site changes can occur, for example, by imparting or translating conformational changes from the second site changes to the CDR binding pocket or to the framework regions. The second site changes can occur in any of the framework regions, including for example, framework regions 1 through 4 as well as in any of the three CDR regions. An advantage of the methods of the invention is that no prior information is required to assess which amino acid positions or changes can be inherently beneficial or detrimental, or which positions or changes can be further augmented by second site changes. Instead, by selecting relevant amino acid positions or subsets thereof in the acceptor variable region framework and CDRs, and generating a diverse population containing amino acid variants at these positions, combinations of beneficial changes occurring at the selected positions will be identified by screening for increased or optimized binding affinity of the CDR graft variable region. Such beneficial combinations will include the

unveiling of inherently beneficial residues, neutralization of inherently detrimental residues and the augmentation of parent residues or functionally neutral changes.

Following selection of relevant amino acid positions in the framework regions and in the donor CDRs as described previously, amino acid changes at some or all of the selected positions are incorporated into encoding nucleic acids for the acceptor variable region framework and donor CDRs, respectively. Simultaneous with incorporating the encoding amino acid changes at the selected positions, the encoding nucleic acids sequences for each of the donor CDRs, including selected changes, are also incorporated into the acceptor variable region framework encoding nucleic acid to generate a population of altered variable region encoding nucleic acids.

An altered variable region of the invention will contain at least one framework position which variably incorporates different amino acid residues and at least one CDR position which variably incorporates different amino acid residues as described previously. The variability at any or all of the altered positions can range from a few to a plurality of different amino acid residues, including all twenty naturally occurring amino acids or functional equivalents and analogues thereof. The different species of the altered variable region containing the variable amino acid residues at one or more positions within the framework and CDR regions will make up the population for which to screen for an altered variable region having binding affinity substantially the same or greater than the donor CDR variable region.

Selection of the number and location of the amino acid positions to vary is flexible and can depend on the intended use and desired efficiency for identification of the altered variable region having substantially the same or greater binding affinity compared to the donor variable region. In this regard, the greater the number of changes that are incorporated into a altered variable region population, the more efficient it is to identify at least one species that exhibits substantially the same or greater binding affinity as the donor. Alternatively, where the user has empirical or actual data to the affect that certain amino acid residues or positions contribute disproportionally to binding affinity, then it can be desirable to produce a limited population of altered variable regions which focuses on changes within or around those identified residues or positions.

For example, if CDR grafted variable regions are desired, a large, diverse population of altered variable regions can include all the non-identical framework region positions between the donor and acceptor framework and all single CDR amino acid position changes. Alternatively, a population of intermediate diversity can include subsets, for example, of only the proximal non-identical framework positions to be incorporated together with all single CDR amino acid position changes. The diversity of the above populations can be further increased by, for example, additionally including all pairwise CDR amino acid position changes. In contrast, populations focusing on predetermined residues or positions which incorporate variant residues at as few as one framework and one CDR amino acid position can similarly be constructed for screening and identification of an altered antibody variable region of the invention.